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Online filing

Sint-Martens-Latem, 02 September 2009

Our ref. : REMY-002-EP-OPP
Re. : European patent no. 1 373 529 granted on April 23, 2008
"Tau-opathy model"
In name of reMynd NV

Dear Madam,
Dear Sir,

Further to your Communication pursuant to R. 79(1) EPC dated March 3, 2009, please find enclosed the proprietor's comments to the opponent's (FoldRx Pharmaceuticals, Inc.) submissions.

Respectfully submitted,

Ms. Paemen, Liesbet
Professional Representative before the EPO

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A. REQUESTS

1. Patentee requests that the patent be maintained as granted and that the opposition be rejected.
2. Patentee requests to be allowed to file auxiliary requests should it be deemed necessary later in the procedure.
3. In case the Opposition Division would consider not to maintain the patent as granted, Oral Proceedings are requested under Article 116 EPC.

B. DOCUMENTS

a) Allowability of the documents cited by the Opponent

The Opponent has submitted a large number of documents. As indicated by the Opponent documents D1-D8 are documents cited as prior art during the international phase and documents D8-D11 were cited during the prosecution of a related U.S. application. Documents D12-D30 are newly introduced documents by the Opponent. It is submitted that documents referred to in the international phase should not automatically form part of the opposition proceedings if no objections have been raised in view of these documents in the Notice of Opposition. The same applies to documents D8-D10 and documents D13 and D18 to D30. None of the arguments presented by the Opponent refer to these documents. It is thus submitted that they should not be considered to form part of the proceedings.

b) New documents referred to in the present submissions

Irrespective of the above, and to avoid confusion, the additional publications referred to in the present document are for the time being numbered consecutively as follows:

Document	Publication date	Author and title
D31	2002	Richardson A. and Burns D. ILAR J. 43(2):89-99. Mouse models of Alzhheimer's Disease: A quest for plaques and tangles.
D32	1984	Kilmartin J. and Adams A. J. Cell Biol. 98: 922-933. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*.
D33	1981	Pringle J. Meth. Enzymol. 194:732-735. Staining of bud scars and other cell wall chitin with calcofluor.

C. THE GRANTED CLAIMS DO NOT CONTAIN ADDED MATTER

As a general note, it is submitted that the Opponent has merely matched the claims as granted with the claims as filed. Moreover, the Opponent has focused on formal aspects of the claim, which are not related to the substance of the claims and as such are mere details which can not be taken as serious arguments. Indeed it appears that some comments of the patentee testify of a hasty and inaccurate reading also of the claims as filed. As is clear from Article 123(2) EPC, the support for the claims must be provided by the application as filed as a whole and need not reside in the precise wording of the claims as filed alone. In what follows, support for each of the claims is nevertheless detailed.

1. Claims 6 and 13

The opponent has argued that the wording of claim 6 and 13 has changed compared to original claim 9 on which it is based, in that it no longer refers to a DNA sequence "encoding a promoter correctly integrated to direct expression of" but rather to a DNA sequence encoding ... under control of a promoter sequence". It is submitted that it is clear to the skilled person that the amendment with regard to the promoter has merely been carried out to improve the clarity of the claim and that a skilled person would have understood that nothing else was intended at the filing date. Moreover the use of an introduced kinase/phosphatase under control of a promoter is clearly supported by the application as filed on page 19, which describes the introduction of PP2A under control of the PGK promoter.

2. claim 7

The Opponent has compared claim 7 as granted with claims 11 and 12 as filed and concludes that claim 7 is unsupported. However, patentee submits that claim 7 represents a combination of claims 8, 9, 10 and 11, all of which refer back to claims 1 to 7. As claims 8 and 9 refer to the introduction of yeast or mammalian kinases respectively, claim 7 is fully supported by the claims as filed.

The opponent furthermore argues that the second and fourth option of claim 7 is unsupported in that the omission of the promoter is not supported by the application as filed. Patentee submits that all of the options recited in claim 7 refer to the presence of a promoter sequence which directs expression of the introduced DNA sequence. Accordingly this objection does not appear to have any basis.

3. Claim 11

The opponent argues that claim 11 is not supported by the application as filed. Patentee submits that the concept of claim 11 is present in claim 19 as filed which refers to modulation which can result in "abolished production of a yeast protein kinase". Indeed, this is further illustrated in the Examples section, where under "general methodologies", the generation of deletion mutants is described (paragraph [0045] of the granted patent). There is no reason why this part of the description could not be used to provide support for the wording of claim 11.

4. Claim 12

The Opponent argues that claim 12 referring to particular mutants of kinases, is only supported by the examples section and as such can not be extrapolated to any yeast. Applicant strongly disagrees. Indeed, as detailed above, claim 19 as filed, in referring back to claim 6 generally envisaged yeast wherein endogenous yeast protein kinase exhibit phosphorylation of the tau protein, which is characterized in that the yeast has been modulated to have increased, decreased or abolished production of a yeast protein kinase. Thus, the application as filed clearly envisaged yeast cells wherein the endogenous kinase genes are modulated. This is exemplified in the examples with specific deletion mutants known in the art. As detailed in the application on page 9, yeast cells in general are envisaged and *Saccharomyces cerevisiae* is a typical embodiment thereof which is selected for its ease in manipulation. Thus, no new subject matter is introduced by the wording of claim 12.

5. Claim 25

The opponent objects to claim 25 in that it refers to the screening of tau function and solubility, while claim 53 as filed on which it is based refers only to tau phosphorylation. Patentee respectfully submits that this argument is again based on a too narrow reading of the claims as filed and moreover ignores the rest of the application. Indeed in fact claim 53 as filed refers to the yeast cell of any one of claims 1 to 40, i.e. including the yeast cell of claim 29 which is a yeast cell which is engineered "for monitoring tau function or tau solubility in a high-throughput screening assay". Accordingly, the method of claim 53 as filed equally envisages detection of tau function and/or solubility. Moreover, Example 7 of the application as filed describes a solubility assay of heterologously expressed tau in yeast. Thus, claim 25 is adequately supported by the application as filed.

6. Claim 32

Opponent asserts that the application as filed provides no formal support for claim 32, which relates to a screening based on the detection specified morphogenic processes in yeast. Patentee strongly objects. Indeed, it is clear that the application as filed envisages high-throughput screening assays which involve screening of morphogenic processes.

Indeed, the general description points out that methods of screening for therapeutic agents are well established and well known and that cell-based methods involve screening of "cell morphological characteristics" (page 16, lines 18-21). Example 6 describes how it was observed that expression of tau in yeast affects physiological processes, which allows the development of high throughput screening assays based thereon. Indeed, example 6 illustrates in figure 6A how expression of tau as such affects pseudohyphal differentiation and benomyl sensitivity. It is thus concluded that heterologous expression of tau affects specified measurable physiological processes. The observed "phenotypes" are a result of expression of heterologous tau, and optionally the protein kinases, present in yeast. The latter are described to be useful in high-throughput screening for compounds which affect various tau-properties including tau-phosphorylation and tau microtubule interaction. It is clear that the application as

filed envisages tau-solubility as one of the properties of tau (Example 7 and throughout application). Accordingly, it is submitted that there is ample support in the application as filed for claim 32.

7. Claim 34

Opponent argues that claim 34 is not fully supported by claim 68 as filed. Patententee again notes that this is objection is based on an overly formalistic approach of the disclosure of the application as filed. Indeed claim 68 as filed referred to an antagonist, *which binds and modulates the activity of an endogenous yeast phosphatase which modulates phosphorylation* of said heterologous expressed tau or isoforms, mutants or functional homologues, *identified using the methods of claims 53 to 67*. Accordingly, this discloses that the methods of the invention can be used in the screening for antagonists *which bind and modulate the activity of an endogenous yeast phosphatase which modulates phosphorylation* of tau. Thus, it is clear that the application discloses the methods of the invention as "methods for identifying an antagonist "which binds and modulates the activity of an endogenous yeast phosphatase which modulates phosphorylation of tau". Again it is thus submitted that the objection against 34 is without real basis.

8. Claim 37

Opponent objects to claim 37 as lacking formal basis in the application as filed. Patentee again disagrees. Claim 37 as granted relates to the use of claim 23 [use of the engineered microbial yeast of any one of claims 1 to 21 for in vivo modeling of tau biochemistry], wherein said tau biochemistry relates to tau aggregation and/or tau microtubule interaction. Support for this claim is found inter alia in claim 74 as filed which relates to:
"*Use of the engineered microbial yeast of any of the claims 1 to 40 as a model for in vivo modeling of protein tau biochemistry comprising tau aggregation and/or tau microtubule interaction.*" Again it is submitted that claim 37 is thus fully supported by the application as filed and the objection is without basis but reflects an incomplete reading of even the claims of the application as filed.

D. THE GRANTED CLAIMS ARE ENTITLED TO THE PRIORITY DATE

The Opponent has presented a vague argument stating that the application is not entitled to the priority date. The Patentee strongly disagrees. However, as none of the novelty arguments presented by the Opponent put this priority date into question, it is submitted that there is no need to address this issue at this time.

E. THE GRANTED CLAIMS ARE NOVEL

The inventors of the patent in suit were the first to demonstrate that it is possible to introduce a DNA sequence encoding tau into yeast, so as to obtain a tau protein which is subject to phosphorylation and dephosphorylation of endogenous yeast enzymes and thus is a functional tau protein. While the prior art may contain statements

generally suggesting the standard recombinant production of protein tau in micro-organisms such as yeast or using tagged tau protein in a two-hybrid screen, it is submitted that this does not anticipate the presently claimed invention.

1. Novelty over D14 (WO 02/065136)

The Opponent has alleged that D14 anticipates claims 1-4, 10, 13-14, 16-17, 19, 20-22, 24-26 and 32-33. Patentee strongly disagrees.

D14 generally describes yeast screens for the treatment of human disease. The diseases envisaged include any human disease potentially involving a misfolded protein, and the list is provided on page 3, lines 7-15. The list of all misfolded proteins envisaged is provided in Table 1 (pages 12-13). In fact the application presents a theoretical catalogue on yeast screens for misfolding diseases, whereby all elements can theoretically be interchanged. Experimental evidence is provided only for the expression of fragments of Huntington and alpha-synuclein in yeast. This can not be considered an enabling disclosure of a yeast expressing tau, let alone for the use of a yeast expressing tau as a model for tau-opathy, or screening methods involving assessment of cell morphology.

2. Novelty over D15

The opponent has alleged that D15 anticipates claims 1-4, 6-8, 10, 13 and 20-21 of the patent in suit. Patentee strongly disagrees.

i. The public availability of D15 before the priority date has not been demonstrated

Patentee notes that, while D15 vaguely refers to "August 2000" on the front page, the Opponent has provided no evidence of the public availability of this document or its content before the priority date. As such, it is questioned whether this document should at all be considered as relevant prior art.

ii. The disclosure of D15 is not enabling

Patentee notes that in the materials and methods section, the author of D15 refers to materials which have been obtained from fellow scientists. More particularly it is noted that for the construction of the tau/pACT2 plasmid, a tau gene is released from a tau/pGEX-T Easy construct made by Dr. Kazuya Sobue. No details of the nature of the tau gene or the construct are provided. Finally it is noted that D15 alleges that no interaction between tau and GSK3 can be demonstrated, while this interaction has been demonstrated to occur in bovine brain. This further questions the reproducibility of the experimental data provided in D15 and more particularly the nature of the tau protein that is allegedly expressed. It is submitted that the experimental data (and more particularly the nature of the tau protein) described in D15 can not be repeated by the skilled person based on the information provided and that D15 can not be considered as an enabling disclosure.

iii. D15 does not disclose the subject matter of the granted claims

Patentee finally notes that the only experiments allegedly carried out in yeast are two-hybrid screens with tau and GSK3ß. For these screens, what is expressed in the nucleus is a tagged tau protein, not tau. Accordingly, D15 does not disclose a yeast cell comprising a sequence encoding tau *per se*, let alone a functional tau protein or phosphorylated isoforms of tau; functional tau is predominantly cytoplasmic in neurons, whilst two-hybrid screens express tau in the nucleus where it is not physiologically functional and thus irrelevant for the subject matter of the granted claims.

3. Novelty over D11 and D12

The Opponent has alleged that claims 1-4, 13-14, and 20-21 are not novel over US5, 492,812 (D11) or WO93/03369 (D12). Patentee disagrees. As both patent documents have a very similar description, for reasons of procedural economy, the arguments presented will refer only to D11, but it will be understood that identical arguments apply to D12.

D11 relates to diagnostic methods for Alzheimer's disease which are based on the detection of proteolytic fragments of the tau protein or auto-antibodies directed thereto (abstract of D1).

In the context of obtaining recombinant tau, D11 theoretically lists a number of methods for obtaining the tau protein, which methods include isolation of material from patient brains and purification of tau proteins, chemical synthesis of tau peptides and cloning and expression of recombinant tau proteins or peptides (item 5.1, columns 7 to 12). It is generally suggested to express a tau protein or fragment thereof in host cells including in a yeast expression vector. The examples however make use of tau isolated from bovine brain. Accordingly it is submitted that the theoretical suggestion of an expression system which could in principle be used for tau (but has not been proven to work for tau) can not be considered as an anticipating disclosure of a yeast expressing tau, let alone expressing a functional tau protein or phosphorylated isoforms of tau.

4. Novelty over D16

The Opponent considers that D16 anticipates the subject matter of claims 1-4 and 18. Patentee does not agree.

D16 is directed to methods and compositions for the diagnosis of tau-opathies. Opponent refers to a section in the description of D16 which theoretically suggests the use of the two-hybrid system to look for proteins that bind to tau. No detail is provided as to how this two-hybrid system is to be performed. In any case, as detailed above, a two-hybrid screen, even if it were disclosed for tau (which is contested), typically involves the expression of tagged proteins and thus do not involve the expression of the tau protein as such. Again, expression in the nucleus does not result in a physiologically functional tau protein or phosphorylated isoforms of tau.

5. Novelty over D17

The Opponent alleges that D17 anticipates claims 1-4 and 20. Patentee contests this statement.

D17 relates to the *in vivo* identification of intracellular epitopes. The application describes the expression in yeast of deletion mutants (in fact corresponding to fragments of the tau protein) fused in frame to a lexA binding domain. The proteins are expressed as fusion proteins, which are then reacted with antibodies with the object of identifying tau epitopes. Accordingly, it is submitted that D17 does not disclose a yeast cell expressing tau.

F. THE GRANTED CLAIMS ARE INVENTIVE

1. The contribution of the invention over the prior art

The inventors of the patent in suit were the first to demonstrate that yeast cells expressing tau in yeast could be used as a model for Tau-opathies, more particularly for Alzheimer's disease.

At the time of the present invention, it was known that the misfolding of phosphorylated protein tau was one of the physiological symptoms of Alzheimer's disease. Accordingly, there was great interest in developing animal or other models which displayed tau misfolding, in order to study the mechanism of the disease and to test potential therapeutics which influence tau misfolding. The generation of a mouse model was not straightforward and the reported models were not always reproducible. In addition, it appeared difficult to obtain a mouse model showing filamentous tau inclusions (neurofibrillary tangles), one of the neuropathological hallmarks of AD and other Tau-opathies. This is apparent, inter alia in the review by Richardson & Burns, 2002 (D31), which states (page 95, left column, second full paragraph: "*Early efforts to produce animal models with tau pathology were based on the expectation that transgenic mouse models of AD that developed amyloid plaques would develop the other classic lesions of AD. However tau-positive neurofibrillary tangles were never observed*"

Given that no established transgenic model was available for studying the tau-associated pathogenesis of Tau-opathies, the invention of a simple yeast-based tau misfolding screen constituted an enormous benefit. Moreover, the yeast-based system of the present invention was the first to allow for high-throughput screening of effects of compounds in countering the development of toxic tau conformations.

Prior to the present invention, yeast cells were generally used as expression systems to generate large quantities of a protein or to study the interaction of proteins in two-hybrid screening.
In the latter type of protein expression in yeast, the protein itself is expressed as a tagged protein in the nucleus and not functional in the yeast cell. While a two-hybrid screen could in theory be designed for all possible protein combinations, the results obtained in practice do not necessarily reflect the *in vivo* interaction (as allegedly demonstrated by D15, see below)

The inventors of the patent in suit were the first to find that tau can be expressed in yeast as a functional phosphorylated tau protein that is prone to aggregate. Accordingly, yeast expressing tau as such could be used as a model for Tau-opathies such as Alzheimer's Disease. This was quite surprising as yeast cells do not naturally express tau or have a nervous system comparable to that of mammals.

This finding was a significant step in the search for new therapeutics as it allowed the screening of compounds in yeast cells directly based on their effect at the physiological level. The applicants were able to develop a screening method which has been solicited by researchers and pharmaceutical companies throughout the world.

2. Failure to demonstrate or suggest the invention by the prior art

The Opponent has not brought any inventive step arguments and has chosen to rely merely on the alleged novelty-destroying documents. As detailed above, none of the documents presented by the Opponent do in fact disclose a yeast cell effectively expressing the tau protein, let alone its use as a model for Alzheimer's disease.

Indeed, all of the references pointed out by the Opponent merely relate to theoretical suggestions of expressing protein tau in yeast (as a bulk protein) or of expressing a tau fusion protein in two-hybrid screening. These documents which in fact merely suggest (and do not effectively illustrate the feasibility of) applying prior art methods on protein tau, do not in any way either alone or in combination disclose or suggest the invention underlying the patent in suit.

It is moreover clear that many of the prior art documents brought forward by the opponent are patent documents which provide a very general theoretical disclosure based on very limited experimental data. While in some instances this may be justified based on a general applicability of the teaching, this is clearly not the case for misfolding proteins or their interactions. Indeed the difficulties of obtaining transgenic models for Alzheimer's disease (D31) and the negative results of D15 illustrate this.

Patentee nevertheless wishes to reserve the right to counter any inventive step argument presented by the Opponent at a later stage, if this would be allowed by the Opposition Division.

G. THE GRANTED CLAIMS ARE SUFFICIENTLY DISCLOSED

Opponent alleges, in item IX of its submissions, that some of the claims are insufficiently supported by the application as filed. Patentee disagrees for reasons detailed hereunder. It is however noted that for most claims, Opponent merely alleges that the claims are insufficiently disclosed, without providing any substantial argument why the skilled person would not be able to carry out the invention as claimed. Accordingly, it is submitted that the objection to these claims is insufficiently supported and should in fact be refused and ignored by the Opposition division.
In order to avoid repetition, a number of the points raised by the Opponent will be addressed together.

1. Claims 4 to 6

Claim 4 relates to a microbial yeast according to claim 1 to 3, wherein endogenous yeast kinases exhibit phosphorylation of tau or an endogenous yeast phosphatase modulates phosphorylation of tau.

Claim 5 relates to a yeast cell according to the invention further comprising an introduced DNA sequence comprising a promoter, correctly integrated to direct the expression of a yeast kinase or phosphatase that modulates the phosphorylation of tau.

Claim 6 relates to a yeast cell according to the invention further comprising a DNA sequence encoding a human or mammalian kinase or phosphatase that modulates the phosphorylation of tau, under control of a promoter sequence.

Opponent considers that claims 4, 5 and 6 are insufficiently supported by the application as filed. However, Opponent admits that the application illustrates the subject matter of claims 4 and 5 for one phosphatase and a number of kinases (5) and discloses the fact that the invention works with a human kinase as claimed in claim 6. It is respectfully submitted that no arguments are provided based on which the skilled person would not be able to carry out the subject of claims 4, 5 or 6 over their entire scope.

2. Claim 7

Claim 7 relates to yeast cells according to the invention further expressing a glycogen synthase kinase-3beta or cdk5 modulating the expression of tau. Again, Opponent admits that the invention is adequately illustrated by two examples and provides no arguments why the invention could not be carried out by the skilled person over the entire scope of claim 7. There is no basis to assume that claim 7 is insufficiently disclosed.

3. Claim 8

Claim 8 relates to a yeast cell according to the invention, wherein the tau protein is co-expressed with an endogenous or exogenous sequence encoding glycogen synthase kinase 3beta-like protein or cdk5-like protein which affects the phosphorylation of tau, whereby tau is phosphorylated, hyperphosphorylated or dephosphorylated.

Opponent states that the application fails to demonstrate hyperphosphorylation of tau and that for this reason the claim lacks enablement.

It is submitted that the examples of the application as filed demonstrate that co-expression of tau with sequences which modulate the expression of endogenous kinases or phosphatases affect the phosphorylation of tau (examples 2, 3 and 4). It is demonstrated that increased expression of kinases induced phosphorylation of tau and that reducing expression of endogenous phosphorylases decreases phosphorylation of tau. The application further demonstrates the aggregation of tau in wild-type yeast

(Example 7). It is known that tau aggregation is only possible when tau is hyperphosporylated.
Thus the application as filed demonstrates that by modulating expression of kinases and phosphatases in yeast, phosphorylation of tau can be modulated, resulting in either phosphorylated, hyperphosphorylated or dephosphorylated tau. Thus, it is submitted that the application as filed provides sufficient support for the subject matter of claim 8.

4. Claims 9 and 31

Claim 9 relates to yeast cells demonstrating phosphorylation, dephosphorylation or hyperphosphorylation of tau as a result of expression of an endogenous or exogenously expressed kinase or phosphatase, wherein at least one further kinase or phosphatase is expressed.

Opponents consider that claim 9 and 31 lack enablement based on the vague statement that "the patent fails to provide technical support for many of the specific kinases and phosphatases to have an effect on tau".

As stated above, the patent demonstrates the effect of different kinases and phosphatases on tau when co-expressed in yeast. More particularly, the patent demonstrates that deletion of mds1 (the yeast GSK3 homologue) results in decreased phosphorylation of the GSK3 sites of human tau expressed in yeast, and that further expression of human GSK3 restores phosphorylation at the GSK3 sites. Thus, this illustrates a yeast strain with decreased phosphorylation as a result of modified expression of an endogenous kinase, wherein a further kinase is expressed, as claimed in claim 9 and 31.

Thus it is submitted that the patent provides sufficient disclosure for the skilled person to carry out the invention as claimed in claim 9 and claim 31.

5. Claim 10 and 11

Claims 10 and 11 relate to specific embodiments of the invention wherein the yeast has been modulated to have modified signal transduction pathways, more particularly where the modulation results in a deletion mutant of an endogenous yeast kinase or phosphatase.

Opponent alleges that it is unclear what signal transduction pathways are intended. Further on, the Opponent admits that claim 11 in fact specifies that the modulation of endogenous signal transduction pathways include the modification of endogenous kinases or phosphatases, but alleges that this is insufficiently illustrated in the patent.

Patentee submits that in the context of the invention, it is clear to the skilled person that the common signal transduction mechanism through phosphorylation and dephosphorylation of proteins is intended. Indeed, the patent in suit teaches that not only modulation of the kinases and phosphorylases directly capable of modulating tau phosphorylation will influence tau phosphorylation, but that these kinases and phosphorylases themselves are modulated by further kinases and phosphatases.

Accordingly, as stated in the patent in suit, the phosphorylation of tau can similarly be modulated indirectly, by acting on these secondary kinases and/or phosphorylases. This is discussed, in the patent in suit, inter alia in the section spanning from page 15, line 16 to page 16, line 5:

"*A very possible factor that could be involved in Alzheimer's disease is hyperphosphorylation of tau. This can be caused by disturbed equilibrium activity of kinases and Phosphatases, which regulate the phosphorylation status of tau. Kinases involved directly in phosphorylation of tau have been identified in vitro by incubation of candidate kinases with recombinant tau produced in bacteria, and have revealed mitogen activated kinases (MAP-kinases) and glycogen synthase kinases (GSK-3ss), cdk5 kinase with any of his activating subunits (p70, p39, p35, p29, p25,...) and other unidentified proline-directed kinases to be capable to phosphorylate tau on epitopes as encountered in PHF-tau (for reviews see Billingsley and Kincaid, 1997, Mandelkow and Mandelkow, 1998; Delacourte, 1999). These are evidently, but not the only candidate kinases that phosphorylate tau in vivo.*

These kinases usually are involved in signaling pathways and require activation by some other mechanism, generally also phophorylation or de-phosphorylation, but eventually proteolytic cleavage.

The mechanisms by which GSK-3ss activity is controlled is by phosphorylation on tyrosine, serine and/or threonine residues by protein kinase C (PKC). MAP kinases are activated by phosphorylation on tyrosine and threonine residues by a MAP kinase (MEK) which is self phosphorylated on serine and threonine residues by MEK kinase (s), possibly identical or related to the proto-oncogenes cRaf-1 or mos, or to the genes Stell and Byr2. These kinases can then be involved indirectly in controlling phosphorylation of tau and thereby used in the present invention to identify candidate kinases and pathways." (emphasis added)

Accordingly, it is submitted that it is clear to the skilled person that the yeast signal transduction pathways referred to in claim 10 relate to the signal transduction through phosphorylation and dephosphorylation of kinases and phosphatases.

As in fact recognized by the Opponent, a specific embodiment of the modulation of this signal transduction mechanism in yeast is provided in claim 11, which refers to a modulation resulting in the deletion of an endogenous kinase or phosphatase.

It is moreover submitted, that contrary to what is alleged by the Opponent, the patent in suit provides not one but several examples of yeast cells wherein the endogenous signaling pathways have been modulated. More particularly, Example 2 discloses yeast cells of strain mds1Δ, lacking one of the yeast genes encoding a kinase homologous to the human tau kinase GSK-3 and yeast cells of strain pho85Δ deficient for a kinase which is homologous to cdk5. Moreover, as pointed out by the Opponent, the patent in suit further discloses yeast cells over-expressing the yeast protein phosphatase PP2A.

Accordingly, it is submitted that the patent in suit provides ample illustration of yeast cells wherein the endogenous signaling pathways have been modulated, including yeast cells wherein this results in deletion of an endogenous kinase or phosphatase.

6. Claim 15

Opponent attacks claim 15 for reasons of lack of clarity, which is not a ground of opposition. As no arguments for lack of enablement are provided, this objection is not addressed.

7. Claim 17

Claim 17 relates to a particular embodiment of the invention, wherein protein tau is coupled in-frame to a reporter protein, wherein tau drives the precipitation of the tau-reporter fusion protein and thereby inhibits changes to the biological function of the reporter protein.

This claim is based on the observation that the aggregation of tau can also be screened using selectable markers, whereby as a result of fusion with tau, the tau-reporter protein is aggregated under the same circumstances, and that screening can be performed based on the reporter protein. This aspect of the invention is described, inter alia, in the section spanning paragraphs [0079] to [0082] of the patent in suit (emphasis added):

"*It can be concluded that tau is predominantly found as insoluble protein in yeast indicating that also in this organism tau forms aggregates and that the aggregation is depending on the phosphorylation status of protein tau. Hence, yeast can be validated as model to study self assembly of tau and to elucidate the formation of paired helical filaments. In addition, such a yeast model offers the opportunity to use tau to drive the aggregation of a selectable marker and as such develop a high throughput screening strategy for components that specifically interfere with tau aggregation.*

Whereas aggregation of Tau is rather time-laborious to monitor, fusion proteins of Tau with enzymatically active proteins appear to show the same phosphorylation-dependent aggregation, and in this case aggregation coincides with the removal of the corresponding enzymatic activity from the cell. This is illustrated by the fusion protein of Tau and the kanamycine-resistance gene product, which is soluble in the mds1d strain (lacking one of the yeast genes encoding a kinase homologous to the human tau-kinase GSK-3ss), but present in an aggregated form in the wild type background. Concomitant with this observation, strains expressing the kanr-Tau fusion in a wild type background are kanamycine sensitive, whereas strains expressing kanr-Tau fusions in the mds1 background are kanamycine-resistant. Any compound inhibiting Mds1 prevents phosphorylation of the kanr-Tau fusion and hence results in kanamycine-resistance.

Hence compounds which induce growth of kanr-Tau fusion expressing wild type cells on kanamycine-containing media are candidate-inhibitors of Mds1. Replacement of Mds1 with its human homologue GSK3, renders this screening assay specific for inhibitors of human GSK3R.

Compounds that cause solubilisation of the kanr-Tau fusion via a mechanism different

from GSK3 inhibition will also yield a positive read-out in this assay. These GSK3independent'Tau solubilisers'are likely to represent interesting new classes of (therapeutic) compounds active against tau-o-pathies. Since monitoring of Tau expression during growth revealed that Tau expression occurred only after 5-10 hours of growth, compounds that reverse Tau aggregation as well compounds that prevent Tau aggregation can be identified depending on the time of administration of the compound to the yeast cultures. Cultures can be grown in microtiter plates and administration of compounds and monitoring of growth (optical density at 600 nm) can be fully automated, allowing the scaling up of the method and the screening of chemical libraries.

The principle of the method described can also be applied to strains expressing fusions of Tau with any other reporter, such as URA3, which has the advantage that functional Ura3 results in growth on medium without uracil, but in toxicity on FOA-containing media allowing a complementary approach and screening.

Accordingly it is submitted that the subject matter of claim 17 is detailed in the patent in suit and that there is no indication from the Opponent why the skilled person would not be able to carry out this aspect of the invention.

8. Claims 19, 25-28 and 30-32

Opponent alleges that claims 19, 25-28 and 30-32 are insufficiently disclosed as the skilled person can not make out what is intended by the term "chemical signal". This is in fact a clarity argument and not a real enablement issue, thus it need not even be addressed. However, it is submitted that the skilled person will understand the term "chemical signal" to generally refer to any signal of a chemical nature (including ions and small molecules). Thus, this term as such would pose no problem to the skilled person for carrying out the invention as claimed.

9. Claim 21

Claim 21 refers to a particular aspect of the invention, which is different from the previous claims in that rather than for use as a model system, yeast cells according to the invention are provided which produce protein tau, suitable for purification and/or production thereof. The reference to the phosphorylation status, merely refers to the different phosphorylation status ensured depending on the embodiment, as specified in claim 8 on which claim 21 depends. It is clear to the skilled person that the requirement "suitable for purification and/or production" essentially refers to a minimal quantity which is desirable.

10. Claims 23 and 37

Claims 23 and 37 relate to the use of engineered yeast cells according to the invention for the in vivo modeling of tau biochemistry.

Opponent alleges that only the phosphorylation and the benomyl-sensitivity of tau are illustrated and thus that the claims are inadequately supported. Patentee disagrees. The inventors of the patent in suit are the first to demonstrate that tau can be

expressed in yeast as a functional protein, i.e. occurring in different phosphorylated isoforms. It is demonstrated that tau is susceptible to the same phosphorylation and dephosphorylation as when expressed in human cells. Moreover benomyl sensitivity is demonstrated. Accordingly, the application as filed provides sufficient credible basis to assume that the model system can be used for testing of all aspects of tau biochemistry. No indication is provided by the Opponent that this would not be the case.

11. Claim 33

Claim 33 relates to a screening method according to the invention based on assessing cell morphogenic processes resulting from the expression of tau in yeast, wherein the specified biological or cell morphogenic processes comprise formation of mitotic bundles, formation of pseudo-hyphen, formation of scar-sites, cell-size, cell metabolism, cell survival or cell growth in defined conditions.

The Opponent alleges that the application as filed provides insufficient guidance as to the effect of tau expression on the formation of mitotic bundles or scar-sites.

Patentee disagrees. Example 6 of the patent in suit demonstrates the effect of different isoforms of tau in yeast on physiological processes. It is demonstrated that there is an effect on pseudohyphal growth and the conditions under which they occur are specified. It is noted that the formation of mitotic bundles and scar-sites in yeast is affected by tau aggregation. Methods for monitoring the formation of mitotic bundles and scar sites during the cell cycle in yeast were known in the art (see inter alia Kilmartin et al. 1984 (D32) and Pringle 1991 (D33). Accordingly, there is no reason to assume that the formation of mitotic bundles and scar sites in yeast could not be monitored in the methods of the invention.

12. Claim 34, 35 and 36

Claim 34 relates to the use of the methods of the invention for the identification of an antagonist which binds to an endogenous yeast kinase, capable of phosphorylating tau.

Claim 35 and 36 relates to methods for identifying structure-function relationship of phosphorylated mutant tau proteins which involve comparing yeast cells wherein a kinase or a phosphatase is co-expressed with tau with yeast cells wherein the kinase or phosphatase is not expressed.

The Opponent alleges claim 34 is lacks support for the same reasons provided against claim 4, i.e., that allegedly only limited guidance on endogenous kinases of yeast kinases is provided. Similarly claims 35 and 36 are objected to based only on the alleged "limited guidance" provided as to which protein kinases and phosphatases affect phosphorylation of human tau.

Again Patentee strongly disagrees and similarly refers to the arguments presented for claim 4. Indeed, it is submitted that the invention is illustrated with different kinases and a phosphorylase and that no argument whatsoever has been provided by the

Opponent which would support the notion that the skilled person would not be able to carry out the invention with other kinases or phosphorylases.

13. Lack of support of the ground of insufficient disclosure (Art. 100(b))

The Opponent's lengthy case on enablement is if at all, based on the vague allegation that there is insufficient teaching on endogenous yeast kinases and phosphatases in the application as filed. As detailed above, Patentee strongly disagrees and considers that this argument not only appears to be based on an incomplete reading of the description, but also attempts to draw the focus of the invention away from the actual contribution of the invention to the art, i.e., the finding that human tau is effectively phosphorylated and dephosphorylated by endogenous kinases and phosphatases in yeast as well as by exogenous enzymes when co-expressed in yeast, thus allowing the use of tau expression in yeast as a model system. No argument whatsoever is provided by the Opponent to demonstrate that the skilled person would not be able to carry out the invention as claimed. Such limited arguments, while nevertheless always requiring, even if only *pro forma*, a rebuttal by the Patentee, should in fact be considered as failing to meet the standard for support of this ground of Opposition and should thus be disregarded by the Opposition Division.

H. CONCLUSION

It has been demonstrated herein that all of the claims of granted patent EP1373529 find basis in the application as filed and thus comply with the requirements of Article 123(2).

It has further been detailed herein that the granted claims are novel and inventive over the cited prior art in that none of the cited documents effectively disclose or even suggest a yeast cell comprising a nucleotide sequence encoding tau per se, i.e. as a functional protein, subject to phosphorylation and dephosphorylation as in the human brain.

Finally, it has been made clear that none of the statements provided by the Opponent can in any way be considered as actual arguments providing basis for the assumption that the skilled person would not be able to carry out the invention as claimed based on the application as filed.

Accordingly, it is respectfully requested that the patent be maintained as granted and that the Opposition be rejected.